airway smooth muscle cells, which is a more physiological system, to determine the mechanism whereby one MLCK phosphorylates many SMM molecules. Direct observations of single QD-labeled MLCK molecules show clearly that MLCK co-localizes with and can move along the actin- and myosin-containing stress fibers, under the conditions of high ionic strength, or physiological ionic strength with CaM-Ca^{2+} and ATP. The diffusion coefficient, calculated from mean-squared-displacement (MSD) data from MLCK-QDs' trajectories, indicates that the mechanism by which one MLCK phosphorylates multiple SMMs may involve MLCK movement along thick and/or thin filaments on a time scale measured in seconds.

1819-Pos Board B589

The Effects of Telokin on the Mechanics of Thiophosphorylated Smooth Muscle Myosin

Genevieve Bates[1], Nedjma B. Zitouni[1], Apolinary Sobieszek[2], Anne-Marie Lauzon[1].
[1]McGill University, Montreal, QC, Canada, [2]Austrian Academy of Sciences, Innsbruck, Austria.

Telokin is a smooth muscle (SM)-specific protein identical to the C-terminal domain of myosin light chain kinase (MLCK). Telokin retards myosin phosphorylation and accelerates its dephosphorylation, but not by simple competition with MLCK. Telokin also prevents myosin's folded conformation. This study explores the effect of telokin on the mechanics of thiophosphorylated SM myosin. We used the *in vitro* motility assay which consists in observing the movement of fluorescently labeled actin filaments as they are propelled by myosin molecules adhered to a microscope coverslip. Four protocols were used: 1) telokin and myosin were incubated together before adhering to the coverslip; 2) to ensure myosin adherence to the coverslip, telokin was added after myosin was adhered; 3) to verify whether telokin affects myosin or actin, telokin was incubated with labeled actin before addition to the myosin adhered on the coverslip; 4) to assess the effects of telokin on actin polymerization, vortexed labeled actin was allowed to repolymerize in the presence of telokin and was then added to the coverslip. Result-1: telokin led to actin filament breakage and thus motility was poor. Difference in filament length was used as a measure of actin breakage. Actin filaments were significantly shorter in the presence of telokin ($3.72\mu m \pm 0.2$ vs. $6.32\mu m \pm 0.98$, n=4, $p<0.05$). Result 2: A non-statistically significant trend towards shorter filaments was observed in the presence of telokin ($3.46\mu m \pm 0.57$ vs. $4.84\mu m \pm 0.3$, n=4, p=0.073). Result 3: No difference in filament length was observed. Result 4: Actin filaments were significantly shorter when repolymerized in the presence of telokin ($1.14\mu m \pm 0.16$ vs. $2.39\mu m \pm 0.44$, n=4, $p<0.05$). In conclusion, telokin acts on thiophosphorylated SM myosin, potentially by altering its conformation, to create strong binding, rigor-like cross-bridges. It also affects actin filament assembly.

1820-Pos Board B590

A Method to Exchange Recombinant Differentially Phosphorylated Rhodamine-Labeled Cardiac RLC into Permeabilized Cardiac Trabeculae

Christopher Toepfer[1], Valentina Caorsi[1], Catherine Mansfield[1], Timothy G. West[2], Thomas Kampourakis[3], Judy Leung[1], James R. Sellers[4], Michael A. Ferenczi[1].
[1]Imperial College, London, United Kingdom, [2]Royal Veterinary College, London, United Kingdom, [3]King's College, London, United Kingdom, [4]NIH, Bethesda, MD, USA.

Phosphorylation of the myosin regulatory light chain (RLC) may be important for regulating the contraction of vertebrate striated muscle fibers. Mutations affecting phosphorylation of the RLC have been shown to correlate with certain familial hypertrophic cardiomyopathies (FHCs), most notably the E22K mutation known to prevent serine-15 RLC phosphorylation. We hypothesise that altered RLC phosphorylation affects both the Ca^{2+} -sensitivity and power output of permeabilized cardiac trabeculae, myofilaments and single myosin molecules. We exchanged expressed non-phosphorylated Rhodamine-labeled cardiac RLC into permeabilized cardiac trabeculae from rat. Temperature jump (T-jump) activation and force-velocity measurements were used to observe changes to mechanical properties of trabeculae. Phos-tag^{TM} SDS-PAGE analysis of left ventricular homogenate from Sprague-Dawley rats determined endogenous RLC phosphorylation levels of 0.44 mol of Pi per mol of RLC. Preliminary force-velocity results using T-jump activation at 20°C of control permeabilized left ventricular cardiac trabeculae (n=5) indicated a peak shortening velocity of 7.9 ± 0.1 FL/s, the shortening velocity at peak power output as 1.8 ± 0.1 FL/s, at which peak isotonic force was measured to be 16.9 ± 1.5% of peak isometric force, 100 ± 9 kN/m^2. We propose to alter phosphorylation of recombinant RLC in-vitro and exchange with native trabecular RLC to see the effects of changing RLC phosphorylation on contractile parameters such as peak power, V_{max} and pCa_{50}.

1821-Pos Board B591

Increased Intrinsic Stiffness of Aortic Vascular Smooth Muscle Cells as a Mechanism for Increased Aortic Stiffness in Spontaneously Hypertensive Rats

Nancy Sehgel[1], Shumin Gao[1], Yi Zhu[1,2], Zhe Sun[2], Jerome P. Trzeciakowski[3], William C. Hunter[1,4], Dorothy E. Vatner[1], Gerald A. Meininger[2], Stephen F. Vatner[1], Hongyu Qiu[1].
[1]Department of Cell Biology and Molecular Medicine, UMDNJ-New Jersey, Newark, NJ, USA, [2]Dalton Cardiovascular Res Center and Department of Medical Pharmacology and Physiology, Univ of Missouri, Columbia, MO, USA, [3]Department of Systems Biology and Translational Medicine, Texas A&M, College Station, TX, USA, [4]Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, NJ, USA.

An increase in vascular stiffness is a fundamental component of hypertension. Our hypothesis is that the increased large artery stiffness in hypertension is due in part to intrinsic properties of vascular smooth muscle cells (VSMCs). Adult spontaneously hypertensive rats (SHR) (16 weeks old) and age-matched Wistar-Kyoto normotensive (WKY) rats were studied. Aortic pressure, measured with a Millar catheter, was higher in SHR than WKY (mean arterial pressure 123 ± 4 vs. 96 ± 6 mmHg). Aortic stiffness, measured with Doppler imaging echocardiography, in vivo, was 2 to 2.5 fold higher, $p<0.05$, in hypertensive rats compared to normotensive rats. Aortic tissue rings and VSMCs were isolated from the SHR and WKY rats and VSMC stiffness was measured, in vitro, in both a reconstituted tissue model and with atomic force microscopy (AFM) in single VSMC's. The continuous force curves with the AFM in single VSMCs were collected and the temporal variation in VSMC elasticity was evaluated by Eigen decompensation. VSMC stiffness was consistently increased by 1.5 to 2 fold in SHR vs. WKY ($p<0.05$). Moreover, the oscillations in elasticity were significantly different between the two groups in terms of frequency and amplitude ($p<0.05$). These observations suggest that not only inherently altered VSMC stiffness, but also unique dynamic elements, are involved in the mechanism of increased aortic stiffness in hypertensive rats.

1822-Pos Board B592

Temporal Analysis of Vascular Smooth Muscle Cell Elasticity and Adhesion Reveals Oscillation Wave Forms that Differ with Aging

Gerald A. Meininger[1], Yi Zhu[1,2], Hongyu Qiu[2], Jerome P. Trzeciakowski[3], Zhe Sun[1], Zhaohui Li[1], Zhongkui Hong[1], Michael A. Hill[1], William C. Hunter[2,4], Dorothy E. Vatner[2], Stephen F. Vatner[2].
[1]Dalton Cardiovascular Res Center and Department of Medical Pharmacology and Physiology, Univ of Missouri, Columbia, MO, USA, [2]Department of Cell Biology and Molecular Medicine, UMDNJ-New Jersey, Newark, NJ, USA, [3]Department of Systems Biology and Translational Medicine, College Station, TX, USA, [4]New Jersey Institute of Technology, Newark, NJ, USA.

In these experiments, a spectral analysis approach was developed for detailed study of time resolved, dynamic changes in vascular smooth muscle cell (VSMC) elasticity and adhesion. Atomic force microscopy (AFM) was used to continuously measure Young's modulus of elasticity and adhesion as assessed by fibronectin or anti-beta 1 integrin interaction with the VSMC surface. Measurements demonstrated that VSMC cells from old versus young monkeys had elevated elasticity (21.6 kPa vs. 3.5 kPa or a 612% elevation in elastic modulus) and adhesion (86 pN vs. 43 pN or a 200% increase in unbinding force). Spectral analysis identified three major frequency components in the temporal oscillation patterns for elasticity (ranging from 1.7x10-3 to 1.9x10-2 Hz in old and 8.4x10-4 to 1.5x10-2 in young) and showed that the amplitude of oscillation was significantly ($p<0.05$) larger in old than in young at all frequencies. It was also observed that patterns of oscillation in the adhesion data were similar to those seen in the elasticity waveforms. In conclusion, these data demonstrate that time resolved analysis of AFM cell elasticity and adhesion measurements provides a uniquely sensitive method to detect functional differences in mechanical and adhesive properties of cells. Aging was observed to increase both elasticity and adhesion in VSMC and also to increase the amplitude and frequency of certain oscillatory components.

1823-Pos Board B593

Small Heat Shock Proteins Associate under Stress Conditions with Elastic Titin Filaments and Provide Protection from Aggregation

Sebastian Kötter[1], Luitgard Nagel-Steger[2], Patrick Lang[1], **Wolfgang A. Linke**[1].
[1]Ruhr Univ. Bochum, Bochum, Germany, [2]Düsseldorf University, Düsseldorf, Germany.

The I-band portion of the giant muscle-protein titin is elastic but detailed knowledge of titin arrangement and interactions in this region is lacking. We determined whether or not select domains from this region can associate with one another, as observed for titin's "distal" immunoglobulin